

Peer Review Report:

Four requests for peer review were sent on 4/24/14. The peer review charge requested review of a specific paragraph and the papers cited in an updated status review (NWFSC 2013) as they pertain to Lolita's heritage and our conclusion that Lolita originated from the Southern Resident killer Whale (SRKW) population.

"As of December 31, 2012, the SRKW population consisted of 84 individuals divided into three pods (26 in J, 19 in K, and 39 in L) (Center for Whale Research and NWFSC unpublished data). An additional captive animal originating from the SRKW population and with a genotype consistent with a southern resident origin (Hoelzel et al. 2007; Hoelzel pers. com.), "Lolita", has resided at the Miami Seaquarium since her capture in August of 1970 (Hoyt 1981). Lolita's original pod is not known with certainty, but her acoustic calls are typical of L pod (Ford 1987; Candice Emmons, personal communication)."

In addition to the peer review request, we provided a letter from Jim Lister on behalf of Miami Seaquarium, dated May 23, 2014, which provided comments on the peer review plan and peer review charge statement for consideration by the peer reviewers. The peer reviewers were also provided with detailed instructions on how to access and search the full set of public comments on the regulations.gov web site.

Two responses were received from

A) A. Rus Hoelzel, Professor of Molecular Ecology, University of Durham

B) David Bain, Ph.D

Below is a combined summary of the two peer reviews, including the main substantive comments received and the conclusions of the peer reviewers about Lolita's heritage and NOAA Fisheries' conclusion that Lolita originated from the Southern Resident killer whale population. We will include and respond to peer review comments in our final rule regarding the petitioned action.

Comments on genetic information

- The SRKW community has a haplotype that is shared by all sequenced individuals within that community (27 whales), and distinct from the haplotypes found in transient, offshore, and northern resident (including whales from SE Alaska and the Bering Sea) communities. (SEE FIGURE 1 of HOELZEL et al. 2007).
- Individuals from the 'west coast transient' population share a single haplotype, as do the 'offshores' and the 'northern residents'.
- Barrett-Lennard (2000: PhD thesis, UBC) screened 126 Northern Resident killer whales and they all shared the same mtDNA haplotype with SE Alaska and the Bering Sea, distinct from the SRKW haplotype.

- Rus Hoelzel was provided with a blood sample for DNA analysis in 1987 by the Miami Seaquarium as cited in publication acknowledgments. This sample was included in population genetic analyses in Hoelzel & Dover (1991), Hoelzel et al. (2002), Hoelzel et al. (2007) and Pilot et al. (2010). All of these papers included investigation of mitochondrial DNA (mtDNA) markers and collectively showed that the MtDNA control region sequence is fixed for a single haplotype within most regional killer whale populations in the North Pacific.
- In the North Pacific the SRKW haplotype is shared only with the whales sampled off the Kamchatka Peninsula in Russia from the Hoelzel dataset, and with AD pod whales from Prince William Sound (PWS) in Barrett-Lennard (2000). Therefore this haplotype is diagnostic indicating natal origin from the SRKW, PWS or Kamchatka Peninsula populations.
- Lolita has the SRKW mtDNA haplotype, and can therefore be assigned to one of these populations on that basis (and excluded from others).
- The potential for Lolita to have originated in the Kamchatka region could be assessed through complete mtDNA sequencing. Morin et al. (2010) found that haplotype in this area could be distinguished from the SRKW haplotype by doing a complete sequence rather than relying on the control region alone.
- Lolita's mtDNA haplotype is unknown in transients.
- Parsons et al. (2013) found no examples of SRKW mtDNA in a sample of 153 transients. It would be more likely that the SRKW haplotype could occur in offshores, given that Hoelzel and colleagues only tested 14 samples (additional samples have been tested in more recent work, such as Morin et al. (2010)).
- While mtDNA was only one of the tests performed, it is very likely to be diagnostic of natal population (Pilot et al. 2009, Morin et al. 2010, Parsons et al 2013).
- Hoelzel et al. (2007) used microsatellite DNA markers to assign clusters and assign kinship. The sample from Lolita could be clustered with other SRKWs based on the program 'Structure,' though this assignment was not unequivocal (there was admixture indicated, though not sufficient to suggest migration or ancestry from any other population (SEE annotation along the top of Figure 3 in Hoelzel et al. 2007). The exact proportional assignments cannot be taken literally, and only indicate relatively weak assignment given the method and data applied.
- Pilot et al. (2010) used an assignment method called "GeneClass", which has more power than the 'Structure' program, and in this case Lolita assigned to the SRKW population with the highest probability (0.464), and with low probability to either the Kamchatka population (0.016) or the SE Alaskan residents (0.004).
- Pilot et al. (2010) used principle components analysis to cluster individuals (PCA; See Figure 1b in Pilot et al. 2010), which again supported the assignment of Lolita to the SRKW population cluster (blue squares in figure) and not with the Kamchatka population (green circles).
- Tests for the assessment of close kin in Pilot et al. 2010 found a putative match between Lolita and the SRKW individual (L97), based on 1 of 4 tests (the other three tests were

unable to resolve kinship in this case). Lolita did not show potential kinship with individuals from any other population.

Acoustic information and morphology

- Ms. Emmon's personal communication regarding the use of L pod vocalizations by Lolita lacks the details necessary to apply it to the question of her population membership.
- Captive killer whales are known to be able to learn the calls of pool mates, even when originating in different ocean basins (Bain 1988). Therefore not knowing where her pool mate Hugo originated from, Lolita's use of L pod calls does not confirm that she is a SRKW. Confirming Hugo was not an L pod whale or that the recordings from Lolita were made before she could learn Hugo's dialect would make it highly likely that she is was an L pod whale therefore a SRKW.
- Recommendation: The date of the cited recording should be determined. If it is more than a year after Lolita was placed with Hugo, it is equally plausible that calls were learned from Hugo as that they were part of her natal dialect. One reviewer suggested analyzing Hugo's genetic material to confirm he did not originate from L pod. This would shed light on whether it was possible for Lolita to learn L-pod calls from him.
- Saddle and dorsal fin shape could be used to further address the question of whether Lolita is a resident or Transient (Bain 1988, Baird and Stacey 1988, Zerbini et al. 2007). There is also evidence that jaw robustness and tooth size vary among Residents, Transients and Offshores which could aid in determining that Lolita is not or is a SRKW.

Capture history and sighting records

- There are no known recorded sightings of any Prince William Sound resident whales overlapping with the range of the SRKW population.
- Location of capture does not rule out that she is a transient (but mtDNA makes this highly unlikely). The capture location makes it highly unlikely that she is a Northern Resident, offshore, Western Pacific, Alaska Resident or from a distant, poorly known population.

Conclusions

- In conclusion it is highly improbable that an immigrant from another population could be as genetically consistent with SRKWs as Lolita is, but additional analysis of Lolita's DNA has the potential to reduce the likelihood further. The joint probability of an immigrant being present at the time of capture who is also genetically similar to SRKWs is far lower.
- Together the mtDNA and the microsatellite DNA data provide a strong case for the assignment of Lolita to the SRKW population, consistent with the historical live capture record. MtDNA data shows fixed differences among populations and are therefore highly diagnostic. The tests using microsatellite DNA data therefore only need to distinguish between putative natal populations that match the mtDNA haplotype, and this is possible with a high degree of confidence for the two relevant populations in the dataset, assigning

- the sample to the SRKW population, and excluding the Kamchatka population (which is of course a geographically improbable source population in any case).
- Although NMFS paragraph supporting Lolita's membership as a SRKW is overly simplistic, it likely correctly concludes that Lolita is a SRKW. None of the location, mtDNA, nor nuclear DNA, rule out her being a SRKW nor make it unlikely.

Additional considerations

- A mtDNA haplotype similar to that found in SRKW was found from the Central Aleutians into Russia (Parsons et al. 2013). One reviewer recommended a thorough genetic analysis could help address uncertainty. A complete mtDNA sequencing or comparing nuclear DNA would address uncertainty. If Lolita had a complete mtDNA sequence consistent with the SRKW haplotype it would rule out membership in any other killer whale population to the degree possible given the sample size for other populations. Expanding the number of microsatellite DNA loci analyzed, e.g., to the 27 employed by Parsons et. al. (2013), and including all available wild SRKW data would clarify the likelihood of patrilineal contributions from other populations.
- If initially A73's genetic information indicated she was a SRKW, but then when rechecked it determined that she was a Northern resident, then Lolita's genetics should also be rechecked. A73 was a unique case, with acoustic calls that matched her pod, her initial mis-identification, and her location which proves that it is possible for a juvenile to stray long distances, therefore, it is important to perform rigorous analyses and double-check results.
- The information to support the determination that Lolita is most likely a Southern Resident should be expanded to both capture the complexity and include a simplified estimate of the relative probabilities that she is or is not a SRKW.
 - NMFS should then evaluate the consequences of treating her as a SRKW in the event that she is and that she is not.
 - NMFS should also consider the consequences of treating her as a non-SRKW in the event that she is and is not.
 - NMFS could decide whether making a mistake could be important enough to consider an additional DNA test, analysis of morphometric data, or trying to track down useful acoustics data. After that NMFS will need to make a decision in the presence of uncertainty, as is commonly the case in management.

References:

Bain, D.E. 1988. An evaluation of evolutionary processes: studies of natural selection, dispersal, and cultural evolution in killer whales (*Orcinus orca*). Ph.D. Dissertation. University of California, Santa Cruz.

Hoelzel, A.R. & Dover, G.A. 1991. Genetic differentiation between sympatric killer whales populations. *Heredity* 66: 191-196.

Hoelzel, A.R., Natoli, A., Dahlheim, M., Olvarria, C., Baird, R.W., Black, N. 2002. Low world-wide genetic diversity in the killer whale (*Orcinus orca*); Implications for demographic history. *Proc. Royal Soc. B* 269: 1467-1475.

Hoelzel, A.R., Hey, J. Dahlheim, M.E., Nicholson, C. Burkanov, V. & Black, N. 2007. Evolution of population structure in a highly social top predator, the Killer whale. *Mol. Boil. Evol.* 24:1407-1415.

Morin, P. A., Archer, F. I., Foote, A. D., Vilstrup, J., Allen, E. E., Wade, P., Durban, J., Parsons, K., Pitman, R., Li, L., Bouffard, P., Nielsen, S.C.A., Rasmussen, M., Willerslev, E., Gilbert, M.T.P. and Harkins, T. 2010. Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. *Genome Res.* 20:908-916.

Parsons, K. M., Durban, J. W., Burdin, A. M., Burkanov, V. N., Pitman, R. L., Barlow, J., Barrett-Lennard, L. G., LeDuc, R. G., Robertson, K. M., Matkin, C. O. and Wade, P. R. 2013. Geographic differentiation among killer whales in the Northern North Pacific. *J. Heredity.* 104:737-754.

Pilot, M., Dahlheim, M.E. and Hoelzel, A.R. 2009. Social cohesion among kin, gene flow without dispersal and the evolution of population genetic structure in the killer whale (*Orcinus orca*). *J. Evol. Biol.* 23:20-31.

Zerbini A.N., Waite J.M., Durban J.W., Dahlheim M.E., Wade P.R. 2007. Estimating abundance of killer whales in the nearshore waters of the Gulf of Alaska and Aleutian Islands using line transect sampling. *Marine Biology.* 150: 1033-1045.